Molecular and Historical Aspects of Corn Belt Dent Diversity

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ABSTRACT

Tens-of-thousands of open-pollinated cultivars of corn (*Zea mays* L.) are being maintained in germplasm banks. Knowledge of the amount and distribution of genetic variation within and among accessions can aid end users in choosing among them. We estimated molecular genetic variation and looked for influences of pedigree, adaptation, and migration in the genetic makeup of conserved Corn-Belt Dent-related germplasm. Plants sampled from 57 accessions representing Corn-Belt Dents, Northern Flints, Southern Dents, plus 12 public inbreds, were genotyped at 20 simple sequence repeat (SSR) loci. For 47 of the accessions, between 5 and 23 plants per accession were genotyped (mean = 9.3). Mean number of alleles per locus was 6.5 overall, 3.17 within accessions, and 3.20 within pooled inbreds. Mean gene diversity was 0.53 within accessions and 0.61 within pooled inbreds. Open-pollinated accessions showed a tendency toward inbreeding (F_{IS} = 0.09), and 85% of genetic variation was shared among them. A Fitch-Margoliash tree strongly supported the distinctiveness of flint from dent germplasm but did not otherwise reveal evidence of genetic structure. Mantel tests revealed significant correlations between genetic distance and geographical (r = 0.54, P = 0.04) or maturity zone (r = 0.33, P = 0.03) distance only if flint germplasm was included in the analyses. A significant correlation (r = 0.76, $P <$ 0.01) was found between days to pollen shed and maturity zone of accession origin. Pedigree, rather than migration or selection, has most influenced the genetic structure of the extant representatives of the open-pollinated cultivars at these SSR loci.

Ten racial complexes of corn have been described in the USA, the economically most important one being the Corn-Belt Dents (Goodman and Brown, 1988). Many Corn-Belt Dents originated in the 1800s from the hybridization of two distinct races of corn, Northern Flints and Southern Dents (Wallace and Brown, 1956). Northern Flint can be traced back to ca 1000 BC in the southwestern USA (Smith, 1995). It spread throughout the Great Plains, moved east of the Mississippi ca AD 600, and was found throughout the eastern USA ca AD 1000 (Brown and Anderson, 1947; Troyer, 2000). The Southern Dents, dominant during colonial times in the southeastern USA, were introduced from Mexican sources via Cuba by Spanish Conquistadors during the 1500s (Doebley et al., 1988; Goodman and Brown, 1988; Hudson, 1994). Dent corn spread northward from Cuba to Florida in 1539, to South Carolina in 1560, and to the Chesapeake Bay area in 1570 (Troyer, 2000).

Because flint corn arrived in the USA 2500 yr before dent, and the two types were isolated for an additional 500 yr by flowering time, they became highly genetically differentiated from each other. Today, Northern Flint and Southern Dent races are considered to be so different that, relative to the variation found within the wild grasses, they would be considered different species and possibly members of different genera (Anderson and Brown, 1952). Flint corn carries certain traits for adaptation to the cool, moist northeastern climate. The typical hard, smooth, and shallow kernels are spaced widely on the ears, allowing them to ripen earlier, be resistant to molds, dry down quickly, and withstand early fall frosts without injury so they germinate well the following spring. This is in contrast to dent types that have kernels arranged compactly on the ear, with deep, rough (dented) grain containing softer starch. On average, flint corn matures earlier than dent corn, but dent corn yields more because of its longer growing season (Jones et al., 1924).

After the American Revolution, the U.S. government began giving away unsettled land or selling it very cheaply, and settlers gradually began moving westward into what is now the U.S. Corn Belt (Hudson, 1994). Corn was not highly valued as a commodity at the time, so it was not the focus of much improvement. In 1814, five *varieties* (now known as *cultivars*) of corn were commonly known: 'Big Yellow', 'Big White', 'Little Yellow', 'Little White' (all flint types) and 'Gourdseed' (white or yellow, the first popular nonflint) (Atkinson and Wilson, 1915). Ten Eyck (1913) claimed that ≈40 corn cultivars were recognized by 1840, and that they were not very distinct from one another. Montgomery (1916) estimated that as many as 250 cultivars were in existence by 1840. The cultivars that were most successful in spreading through the Corn Belt traced back to early crosses between flint and dent types. The most famous example of this is illustrated by the story of Robert Reid, who serendipitously crossed a semi-gourdseed dent with Little Yellow in Illinois in 1847 to create 'Reid Yellow Dent' (Wallace and Brown, 1956; Troyer, 1999). The hybrid origin of Corn-Belt Dent

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Abbreviations: CI, confidence interval; QTL, quantitative trait loci; RFLP, restriction fragment length polymorphism; SSR, simple sequence repeat.

cultivars did not generate much discussion in late nineteenth and early twentieth century agricultural reports. This is understandable because corn breeding efforts were generally ignored in early nineteenth century agricultural literature (Atkinson and Wilson, 1915).

From 1870 to 1880, the free lands of the eastern Dakotas, Nebraska, and Kansas were exhausted, and more attention was given to improving corn (Atkinson and Wilson, 1915). In 1887, the U.S. Agricultural Experiment Stations were established, and efforts were directed nationwide toward corn improvement. By 1890, farmers were being urged to practice selection and fix favorable types for local growing conditions. Production of sufficient seed corn for the newly settled lands was seen as problematic, and it was recognized that farmers could increase yields if they grew adapted and proven cultivars (Hays, 1890). In 1901, 35 240 m^3 of seed corn were required for annual planting in Illinois (Shamel, 1901). At that time, it was reported that mostly *mongrel* cultivars were being grown (Shamel, 1901). These were not selected toward a particular type or for any special purpose. Approximately 800 North American cultivars were described in 1899. Many of these were synonyms, but no details were provided on pedigree (Sturtevant, 1899). There were probably closer to 1000 cultivars at that time, as Sturtevant would not have been able to secure them all (Montgomery, 1916). The Illinois Seed Corn Breeders' Association was formed in 1900 at the University of Illinois with the stated purpose of providing good seed corn for planting. A corn registry was begun to trace pedigrees for breeding purposes as was done with livestock. Extensive efforts were put forward for standardization of corn cultivars by experiment stations and corn growers' associations in the early decades of the twentieth century (Atkinson and Wilson, 1915; Cox and Duncan, 1920).

In ≈1920, the inbred-hybrid method of corn breeding commenced. Open-pollinated cultivars were rapidly replaced by hybrids obtained by crossing highly-inbred lines (Anderson, 1944). The first hybrid seed for sale (Burr-Leaming double cross) was produced at Clinton, CT, by G.S. Carter in 1921 (Jenkins, 1936). In 1933, <1% of the Corn Belt was planted to hybrids (Hallauer and Miranda, 1988). By 1936, the USDA discussed corn cultivars as an important natural resource and source of inbred lines (Jenkins, 1936). It was difficult to find a farmer's field of open-pollinated corn by the 1940s (Anderson, 1944; Jones and Everett, 1949). The potential narrowing of the germplasm base was immediately recognized, as it was pointed out that six popular double-cross hybrids traced their ancestry back to only two open-pollinated populations (Anderson, 1944). Ninety-six percent of the U.S. corn hectarage was planted to hybrids by 1961 (Hallauer and Miranda, 1988). Hybrid seed corn production has been dominated by private industry since the 1930s. Today, private industry is the source for virtually all of the planted hectarage of U.S. corn, with only a few farmers still growing open-pollinated populations (F. Kutka, 2000, personal communication).

Recognizing that genetic uniformity of crops could lead to susceptibility and yield loss through pests or climatic conditions, efforts were made beginning in the 1950s to collect historically important open-pollinated corn populations and deposit them into the U.S. National Plant Germplasm System for conservation. Because much of the germplasm was assembled based on phenotype and geographic origins, little evidence exists concerning genotypic variation or detailed relationships among accessions (P.K. Bretting, 1999, personal communication). Occasionally, some of the unimproved accessions are tested for their potential to contribute superior traits to commercial germplasm (Salhuana et al., 1998). The narrow genetic base of U.S. hybrid corn is still a concern, with the relative proportion of Reid Yellow Dent-derived germplasm in modern hybrids estimated to be 50% (Troyer, 1999).

Given the number of accessions currently available in germplasm banks, evaluation is difficult. Knowledge of how molecular genetic variation is partitioned within and among accessions of conserved Corn-Belt Dent germplasm provides a framework from which end users can choose accessions for evaluation based on their genetic relationships. To understand the underlying basis of genetic structure, one must consider the influences of pedigree, migration, and selection. The purpose of this study was to use SSR markers (i) to test whether there is genetic differentiation between accessions of traditional open-pollinated Corn-Belt Dent cultivars; this pertains to how much admixture between cultivars has occurred, (ii) to test for isolation-by-distance, that is, whether genetic distance between accessions is significantly correlated with physical (km) distance or climatic (maturity zone) differences between their sites of origin; these can be related to migration and selection, respectively, and (iii) to examine whether or not genetic relationships reflect pedigrees, to the extent that pedigrees are known.

MATERIALS AND METHODS

Germplasm

We genotyped 461 plants representing a diverse array of Corn-Belt Dent-related germplasm (44 open-pollinated Corn-Belt Dents, 8 Northern Flints, 4 Southern Dents, 1 synthetic, and 12 inbreds; Table 1) at 20 SSR loci (Table 2). Origins of genotyped materials were compiled from the literature and from GRIN (Germplasm Resources Information Network, http://www.ars-grin.gov/) (Table 1). Seed was obtained from the North Central Regional Plant Introduction Station in Ames, IA, or from Dr. Arnel R. Hallauer's and Dr. Kendall R. Lamkey's breeding programs at Iowa State University; DNA of inbreds B73 and Mo17 was obtained from Pioneer Hi-Bred International, Inc., Johnston, IA. Between 1 and 25 seeds were planted per accession. For Northern Flint and Southern Dent, we sampled one plant per accession because they were used as outgroups. An outgroup is defined as one or more taxa that lie outside the group in which we are trying to detect relationships. For 40 accessions, we sampled eight plants and genotyped between five and eight plants each (Table 3). For seven accessions, we sampled 25 plants and genotyped between 19 and 23 plants (Table 3); this was because we wanted to see if mean number of alleles substantially

Table 1. Historical origins of Corn Belt Dent accessions (originator, geographical location, approximate year, and pedigree if known), and PI number and origin of strain that was genotyped.

Entry	Name	Historical origin	Reference	PI number	Strain origin
1	Bloody Butcher	Synonymous to King Philip, Strawberry. Generic name applied to dark-red-hulled types.	Sturtevant, 1899; Wallace and Bressman, 1937, p. 217–218	218195	Rector strain, Wood Co., WV
2	Bowman's Cole Creek	Synonymous to Kansas No. 80, grown in Lebo, KS, since 1874.	USDA, ARS, NGRP, 2002	222635	Lebo, KS
3	Clarage	E. Clarridge, Duffs Fork, Fayette Co. OH, 1813, selected from local corn.	Abbott, 1911; Troyer, 1931	278713	Eichelburger strain, OH
4	Clarage			278721	Wooster strain, northern OH
5	Dawes #2	Possible selection of Minnesota 13.	USDA, ARS, NGRP, 2002	222311	Western NE
6	Early Butler	Synonymous to King of the Earliest.	Sturtevant, 1899	217473	Grand Valley, PA
7	Falconer	A. Falconer, Bismarck, ND, 1887, from Indian flint × yellow dent brought by a pioneer.	Olson et al., 1927	213781	ND
8	Fulton Yellow Dent	H.E. Dawes and H. Thompson, Fulton, Hanson Co., SD. Selected from Shabbino corn from WI.	Hume, 1923	213780	ND
9	Funks Yellow Dent	E.D. Funk, Sr., McLean Co., IL, 1901, selected from original Reid Yellow Dent.	Wallace and Bressman, 1937, p. 216–217; Troyer, 1999	213696	Lyndon, IL
10	Golden Glow	WI, Minnesota13 × Toole's North Star.	Hutchison et al., 1916; Gerdes et al., 1993, p. 128	213722	Emmons, MN
11	Golden Glow	Very early strain of Golden Glow developed by J. Smithers, Marian, MI.	USDA, ARS, NGRP, 2002	222469	Smithers strain, Marian, MI
12	Golden Glow			280852	Southern WI
13	Golden Republic	Uncommon, flinty type from north central Kansas.	Lonnquist and Gardner, 1961	222318	South central NE
14	Hays Golden	Hays, KS, experiment station.	Lonnquist and Gardner, 1961	222315	Southwestern NE
15	Iowa Ideal	H. Hilton, Malvern, IA, 1894, obtained local variety named St. Charles White and renamed it Iowa Ideal after improvement.	Bowman and Crossley, 1911, p. 443	278723	Indianola, IA
16	Krug	G. Krug, Woodford Co., IL, obtained a NE strain of Reid Yellow Dent in 1903. Three years later this was combined with Goldmine and an IL strain of Reid Yellow Dent.	Hughes et al., 1929	213699	Steamboat Rock, IA
17	Krug			213708	Johnston, IA
18	Krug			213717	From G. Krug, Jr., Minonk, IL
19	Krug			222316	Shroup strain, east central NE
20	Lancaster Sure Crop	J.E. Hershey, Lancaster, PA, 1868, mixed seed from U.S. Patent Office obtained in 1860 by H. High, Byerston, PA, with local corn.	Troyer, 1999	213697	Lancaster, PA
21	Lancaster Sure Crop			280061	OH
22	Leaming	J.S. Leaming, Wilmington, OH, 1826 or 1855, from ordinary 'little yellow' corn grown on bottomlands of Little Miami River, Hamilton Co.; Synonymous to Pride of the North.	Shamel, 1901; Abbott, 1911; Hutchison et al., 1916; Sturtevant, 1899	278717	Central and southern OH
23	Legal Tender	Nims Bros., Emerson, IA, 1876, from crossing two distinct varieties.	Bowman and Crossley, 1911, p. 434–436	213716	Clarion, IA
24	Little Briton	N.R. Funk, Carbondale, IL, in family for at least 100 years.	USDA, ARS, NGRP, 2002	414176	Carbondale, IL
25	Longfellow	S. Longfellow, Newbury, MA, 1877, from New England 8-rowed flint 'King Philip' and two other varieties.	Atkinson and Wilson, 1915	214195	Ontario, Canada
26	Midland	O.A. Rhoads, Columbus, Cherokee Co., KS, 1884, from local yellow corn of unknown origin.	Cunningham and Wilson, 1921	213712	Malvane, KS
27	Midland			213725	El Dorado, KS
28	Midland			222609	Columbus, KS
29	Midland			222615	Oswego, KS
30	Minnesota 13	Minnesota Experiment Station obtained seed in 1893 from DeCou & Co. and invoiced it as #13. Originated from common corn grown in St. Paul area.	Atkinson and Wilson, 1915; Troyer, 1999	214197	Ontario, Canada
31	Northern Wonder	No reference		217475	Hackettstown, NJ
32	Osterland	H.F. Osterland, Franklin Co., IA, 1915. Original stock from Reid Yellow Dent, Wright Co.	Hughes et al., 1929; Gerdes et al., 1993, p. 131	213721	Sac City, IA
33	Perkins Yellow Dent	Perkins family, Adair Co., MO.	USDA, ARS, NGRP, 2002	269744	Adair Co., MO

Continued on next page.

Table 1. Continued.

Entry	Name	Historical origin	Reference	PI number	Strain origin
34	Pickett	J.W. Pickett, Caledonia, Kent Co., MI, 1890; secured seed from W.E. Boyden of Delhi Mills, MI. Originated from extremely early variety of Reid Yellow Dent obtained from northern IL in 1885 by Michigan Ag. Expt. Station.	Cox and Duncan, 1920	222470	Bloomington, MI
35	Polar Dent	Strain of Duncan adapted to southern MI.	USDA, ARS, NGRP, 2002	222474	Mason, MI
36	Pride of Saline	C.H. Kellogg, Russell Co., KS, 1891, secured seed from B. Bradt of Gorham, KS. From local white corn, possibly Silvermine or White Pearl.	Ten Eyck, 1913	214295	KS
37	Reid Yellow Dent	R. Reid, Delavan Plains, IL, 1846, from Gordon Hopkins seed from Ohio × Little Yellow, a local Indian flint type.	Troyer, 1999	213698	Springport, IN
38	Reid Yellow Dent			213705	Buffalo, IL
39	Reid Yellow Dent			213709	Clear Lake, IA
40	Reid Yellow Dent			213719	Elsberry, MO
41	Reid Yellow Dent			222317	Nubold strain, central NE
42	Reid Yellow Dent			222613	Kansas
43	Reid Yellow Dent			408705	Grand River, IA
44	Silver King	H.J. Goddard, Ft. Atkinson, IA, 1904, from a corn brought to IA from IN in 1862.	Hutchison et al., 1916; Hughes et al., 1929; Cox, 1921	280853	Southern WI
45	West Virginia Clarage	Same as Clarage (see 3 above)		218005	Willow Bend, Monroe Co., WV
46	Woodburn	J.D. Woodburn, Urbana, OH, 1891, crossed local yellow corn (possibly Leaming) to an early maturing corn from the Ohio River, east of Cincinnati.	Bowman and Crossley, 1911	278720	OH
47	Iodent	Traces to a remnant ear of Reid Yellow Dent showing early maturity, Ames, IA, 1915, improved by ear-to-row and mass selection.	Hughes et al., 1929; Wallace and Bressman, 1937		ISU†
48	Assiniboine	Assiniboine Tribe, Canada.	Dunham, 1928	213793	ND
49	Cheyenne (Concho Brown)	Cheyenne Tribe, OK.	USDA, ARS, NGRP, 2002	213748	OK
50	Gaspé	Gaspé Village farmers, Quebec, Canada.	González Ugalde, 1997	214279	Not found in GRIN
51	Longfellow	New England 8-rowed flint.	Atkinson and Wilson, 1915	217408	IA
52	Sioux (Creek)	Sioux Tribe, SD.	USDA, ARS, NGRP, 2002	213770	SD
53	Winnebago (Yankee Cheat)	Winnebago Tribe, NE.	USDA, ARS, NGRP, 2002	213771	NE
54	Gourdseed	Native to TX.	Hartley et al., 1912	213715	TX
55	Jellicorse	R. Jellicorse, Elmwood, Smith Co., TN.	Mooers, 1922	452046	TN
56	Tennessee Red Cob	Originally from TN.	USDA, ARS, NGRP, 2002	311235	VA
57	White Dent	No reference		221885	Marshall, Saline Co., MO
58	Wf9	Purdue Wilson Farm Reid Yellow Dent	Baker, 1984		ISU
59	Mo17	CI187-2‡ × C103§	Baker, 1984; Gerdes et al., 1993, p. 4, 62		Pioneer Hi-Bred Int'l, Inc.
60	A682	[(AS-D¶ × Mo17)Mo17(2)]	Gerdes et al., 1993, p. 36, 104		ISU
61	A683	[(AS-D¶ × Mo17)Mo17(2)]	Gerdes et al., 1993, p. 36, 104		ISU
62	C103	Lancaster Sure Crop	Gerdes et al., 1993, p. 4		ISU
63	Pa91	(Wf9 × Oh40B§)S_4 × [(38-11# × L317§)38-11#]S_4	Gerdes et al., 1993, p. 18, 24, 53		ISU
64	Oh43	W8†† × Oh40B§	Gerdes et al., 1993, p. 10, 51, 65		ISU
65	B14A	Cuzco × B14(8)‡‡	Gerdes et al., 1993, p. 21, 101; Troyer, 1999		ISU
66	B73	Iowa Stiff Stalk Synthetic C5	Gerdes et al., 1993, p. 22		Pioneer Hi-Bred Int'l., Inc.
67	A680	[(A662§§ × B73)B73(3)]	Gerdes et al., 1993, p. 36, 104		ISU
68	B37	Iowa Stiff Stalk Synthetic	Gerdes et al., 1993, p. 22		ISU
69	CI 540	Pioneer TEA54 (H.A.Wallace), from Ill. 2-ear	Gerdes et al., 1993, p. 62		ISU

† Cooperative Federal-State Maize Breeding Program, Iowa State University.
‡ From Krug.
§ From Lancaster Sure Crop.
¶ Synthetic from inbred lines A73, B14, CO106, ND255, Oh43, V3, WD, and Wf9; these inbred lines were derived at least in part from Golden Glow, Iowa Stiff Stalk Synthetic, Minnesota 13, Lancaster Sure Crop, Reid Yellow Dent, and Wisconsin No. 25.
# From 176A.
†† M13 × Ill.A48, from Minnesota 13 and from Funks Yellow Dent, respectively.
‡‡ From Iowa Stiff Stalk Synthetic, a synthetic of 16 inbred lines of which at least 10 originated from Reid Yellow Dent.
§§ From Minnesota Synthetic AS-A, synthesized from inbred lines A90, A498, A508, A509, A513, CMD5, MS1334, ND203, W33, W59M, W79A, W65, and W103; these inbred lines were derived at least in part from Northwestern Dent, Rustler White Dent, Minnesota 13, Silver King, Holbert Yellow Dent, Golden Glow, Maize Amargo, and Wisconsin No. 25.

Table 2. Twenty SSR markers used to Genotype 461 corn plants representing 57 accessions and 12 inbred lines.

Marker	Multiplex set	Locus	No. of alleles	Size, bp		Map location	Repeat size	Label†	Missing data
				Min.	Max.				
1	A	phi022	8	368.89	396.77	9.03	4	6-FAM	0.0023
2	A	phi115	3	292.21	292.59	8.03	6	HEX	0.0003
3	A	phi033	8	236.98	263.64	9.02	3	6-FAM	0.0001
4	A	phi079	5	180.92	193.09	4.04	5	TET	0.0027
5	A	phi062	2	160.78	164.09	10.04	3	HEX	0.0001
6	A	phi051	6	138.69	147.54	7.06	6	6-FAM	0.0037
7	A	phi127	4	112.12	126.56	2.07	4	TET	0.0004
8	A	phi015	11	83.29	104.80	8.08	4	HEX	0.0000
9	B	phi093	11	284.84	295.77	4.08	4	TET	0.0007
10	B	phi085	7	229.68	262.03	5.06	5	6-FAM	0.0010
11	B	phi053	7	166.73	212.88	3.05	4	TET	0.0010
12	B	phi072	7	142.36	162.95	4.01	4	6-FAM	0.0001
13	B	phi034	10	116.77	145.37	7.02	3	HEX	0.0001
14	B	phi121	2	98.02	101.66	8.04	3	6-FAM	0.0000
15	C	phi056	6	232.77	264.15	1.01	3	HEX	0.0017
16	C	phi032	3	234.51	242.71	9.04	4	TET	0.0028
17	C	phi011	7	209.27	230.52	1.10	3	6-FAM	0.0013
18	C	phi090	4	141.03	151.28	2.08	5	HEX	0.0004
19	C	phi083	6	125.69	137.78	2.04	4	TET	0.0000
20	C	phi064	13	77.28	113.80	1.11	4	HEX	0.0000

† 6-FAM, 6-carboxyfluorescein; TET, tetrachloro-6-carboxyfluorescein; HEX, hexachloro-6-carboxyfluorescein.

increased with an approximate three-fold increase in sample size. When fewer plants were genotyped than sampled from an accession, it was because of poor DNA quality. One plant per inbred line was sampled because we assumed that an inbred would be genetically uniform. DNA was extracted from ≈50 mg freeze-dried tissue using a CTAB miniprep (Mitchell et al., 1997). Fluorescently labeled polymerase chain reaction (PCR) primers were synthesized at the Iowa State University DNA Sequencing and Synthesis Facility. All primer sequences have been published (Smith et al., 1997) and are available on the MaizeDB (http://www.agron.missouri.edu/). We genotyped 20 SSR loci randomly distributed across the corn genome (Table 2). Eight additional loci (*phi006*, *phi014*, *phi024*, *phi041*, *phi070*, *phi113*, *phi078*, and *phi050*) were amplified and scored but excluded from any analyses because >5% of the data were missing, either from null alleles, failed amplifications, or scoring inconsistencies. Polymerase chain reaction and electrophoresis on an automated DNA sequencer (Model 377, PE Biosystems, Foster City, CA) were performed as in Matsuoka et al. (2002). Raw data were scored using PE Biosystems' GeneScan v. 2.1 and Genotyper v. 3.0 software.

The selective criteria used to pick the accessions used for Mantel isolation-by-distance tests were that the accessions (i) had written accounts of time and place of origin, (ii) were popular in the early 1900s, and (iii) were treated as distinct types at that time. Eleven accessions met these criteria, Entries no. 3 Clarage, no. 7 Falconer, no. 20 Lancaster Sure Crop, no. 22 Leaming, no. 23 Legal Tender, no. 25 Longfellow, no. 28 Midland, no. 30 Minnesota 13, no. 36 Pride of Saline, no. 38 Reid Yellow Dent, and no. 44 Silver King (Table 1). The original cultivar was chosen to represent strains that were thought to be closely related (a strain being a population of a cultivar that is adapted to a particular environment). For example, the Illinois strain of Reid Yellow Dent was included because Reid Yellow Dent originated in Illinois; and all other Reid Yellow Dents, as well as all known Reid Yellow Dent derivatives, were excluded. Any accession of unknown origin was excluded. Most of the accessions used in the Mantel tests included an account of gene flow in their descriptive history (Table 1), often the mixing of pioneer-introduced corn with local corn.

Pollen shed data for 27 of the accessions were available and taken from GRIN (http://www.ars-grin.gov/cgi-bin/npgs/html/eval.pl?491302). These data were collected by the Latin American Maize Project (LAMP) 10 01 Ames 1987, and are defined as number of days from planting to when 50% of observed plants have shed pollen.

Statistical Analyses

Descriptive statistics and *F*-statistics were estimated using GDA 1.0 software (Lewis and Zaykin, 1999). *F*-statistics are used to describe the distribution of genetic variation within a hierarchical framework. Prevention of gene flow results in an apparent deficiency of heterozygosity relative to random mating. This can be expressed as a mean reduction in expected heterozygosity within one level of a hierarchy because of nonrandom mating at a higher level of the hierarchy. The hierarchical structure of the open-pollinated accessions can be viewed as individuals within accessions, groups of accessions with identical names, and a total group containing all accessions.

F-Statistics were estimated in two ways. In the first case, the mean amount of heterozygosity within accessions (Table 1, Entries 1 to 47) was compared with the amount of expected heterozygosity within the pooled group of 47 accessions. In the second case, only accessions, for which there was more than one entry with the same name, were included in the analyses. This consisted of 22 accessions placed into six groups: Clarage (Table 1; Entries 3, 4), Golden Glow (Table 1, Entries 10–12), Krug (Table 1, Entries 16–19), Lancaster Sure Crop (Table 1, Entries 20–21), Midland (Table 1, Entries 26–29), and Reid Yellow Dent (Table 1, Entries 37–43). To generate 95% confidence intervals (CIs) of *F*-statistics parameters, 1000 bootstrap replicates across loci were performed using GDA.

PHYLIP v 3.57c (Felsenstein, 1995) was used to construct a Fitch-Margoliash tree (Fitch and Margoliash, 1967). The Fitch-Margoliash method uses genetic distances between pairs of taxa (i.e., accessions) to construct a tree that best matches the data, without assuming a molecular clock. The Fitch-Margoliash method is useful with distances computed from allele frequencies (Felsenstein, 1995). A consensus tree based on 100 bootstrap replicates of allele frequency data was obtained using Reynolds et al. (1983) genetic distance:

$$D = \frac{\sum_m \sum_i (p_{1mi} - p_{2mi})^2}{2\sum_m (1 - \sum_i p_{1mi}\, p_{2mi})}$$

for m loci and i alleles, where p_{xmi} is the frequency of the ith

Table 3. Mean descriptive statistics for genotyped corn accessions across 20 SSR loci.

Entry†	Name	Sample size	Sample size corrected for missing data	No. of alleles	Gene diversity	Observed heterozygosity	Coefficient of inbreeding	Days to pollen shed‡	Maturity zone
1	Bloody Butcher	7	6.80	3.10	0.50	0.44	0.13	70	7
2	Bowman's Cole Creek	8	7.90	3.60	0.61	0.47	0.23	80	8
3	Clarage	8	7.90	3.60	0.62	0.57	0.09	–	6
4	Clarage	7	7.00	3.25	0.56	0.51	0.10	–	6
5	Dawes #2	8	8.00	3.40	0.54	0.54	0.00	64	4
6	Early Butler	6	5.90	2.80	0.54	0.51	0.07	62	3
7	Falconer	7	6.80	3.60	0.60	0.45	0.25	–	2
8	Fulton Yellow Dent	7	6.90	2.90	0.50	0.41	0.19	–	2
9	Funks Yellow Dent	6	6.00	2.85	0.52	0.44	0.16	70	5
10	Golden Glow	7	6.80	3.45	0.57	0.49	0.15	68	4
11	Golden Glow	7	6.80	3.25	0.55	0.49	0.12	–	2
12	Golden Glow	7	6.85	3.40	0.59	0.51	0.14	66	4
13	Golden Republic	7	6.65	3.25	0.59	0.54	0.08	70	7
14	Hays Golden	7	6.90	3.25	0.54	0.46	0.16	68	5
15	Iowa Ideal	8	8.00	3.35	0.56	0.51	0.09	73	7
16	Krug	21	20.95	3.55	0.52	0.50	0.05	71	5
17	Krug	7	7.00	3.35	0.55	0.58	−0.06	–	6
18	Krug	8	8.00	3.55	0.58	0.57	0.02	70	6
19	Krug	8	7.95	3.50	0.60	0.60	0.01	72	7
20	Lancaster Sure Crop	8	7.70	2.95	0.54	0.51	0.06	68	7
21	Lancaster Sure Crop	20	19.50	3.45	0.52	0.46	0.12	67	6
22	Leaming	8	7.80	3.15	0.53	0.51	0.04	63	8
23	Legal Tender	8	7.70	3.10	0.48	0.46	0.06	70	5
24	Little Briton	7	6.80	3.10	0.52	0.49	0.06	70	8
25	Longfellow	22	21.10	2.65	0.33	0.26	0.22	61	2
26	Midland	22	21.70	3.70	0.52	0.49	0.06	–	8
27	Midland	8	7.80	2.80	0.48	0.40	0.18	–	8
28	Midland	8	7.85	2.75	0.48	0.48	0.01	81	8
29	Midland	7	6.90	3.25	0.55	0.51	0.06	–	8
30	Minnesota 13	8	7.95	2.65	0.46	0.40	0.13	56	2
31	Northern Wonder	8	7.90	2.60	0.44	0.39	0.12	–	6
32	Osterland	8	7.95	2.45	0.44	0.44	−0.02	–	6
33	Perkins Yellow Dent	8	7.90	3.15	0.59	0.55	0.06	71	7
34	Pickett	6	5.95	2.95	0.51	0.50	0.02	–	5
35	Polar Dent	7	6.90	2.85	0.53	0.38	0.30	–	4
36	Pride of Saline	5	5.00	2.65	0.45	0.41	0.11	78	8
37	Reid Yellow Dent	20	19.55	3.40	0.48	0.42	0.14	71	7
38	Reid Yellow Dent	7	6.80	3.40	0.60	0.54	0.11	74	7
39	Reid Yellow Dent	8	7.80	3.45	0.57	0.54	0.05	–	5
40	Reid Yellow Dent	6	6.00	3.00	0.57	0.54	0.05	–	8
41	Reid Yellow Dent	8	7.80	3.30	0.55	0.55	−0.01	–	6
42	Reid Yellow Dent	7	7.00	2.95	0.51	0.42	0.18	75	8
43	Reid Yellow Dent	7	6.80	2.75	0.52	0.43	0.18	–	7
44	Silver King	8	7.80	3.65	0.58	0.51	0.12	67	4
45	West Virginia Clarage	23	22.45	3.75	0.54	0.50	0.08	–	8
46	Woodburn	7	7.00	3.10	0.56	0.49	0.14	–	6
47	Iodent	19	18.60	3.05	0.52	0.50	0.04	–	6
48§	Northern Flints	6	5.65	2.60	0.42	n.a.¶	n.a.	n.a.	n.a.
49#	Southern Dents	4	3.80	2.85	0.54	n.a.	n.a.	n.a.	n.a.
50††	Inbreds	12	11.80	3.20	0.61	n.a.	n.a.	n.a.	n.a.
	Mean (Entries 1–47)	9.3	9.17	3.17	0.53	0.48	0.10		

† Entries also refer to Table 1.
‡ Data from GRIN, Latin American Maize Project 10 01 Ames 1987, dash indicates not available.
§ One plant from each of Entries 48–53 in Table 1.
¶ Not applicable because these samples were pooled from different accessions.
# One plant from each of Entries 54–57 in Table 1.
†† One plant from each of Entries 58–69 in Table 1.

allele at the *m*th locus in population x. This distance measure is appropriate when divergence between populations is primarily due to drift rather than mutation (Weir, 1996, p. 195).

GENEPOP software 3.1d (Raymond and Rousset, 1995) was used for Mantel tests to look for a relationship between molecular genetic distance and physical (km) or climatic (maturity zone) distance between pairs of accessions according to their sites of origin. Maturity zones were numbered two through eight (Table 3), distance between a pair of maturity zones was expressed as their absolute difference. The observation that genetic distances between pairs of populations increases with increasing physical distance is known as *isolation-by-distance*. It implies that gene flow among a contiguous set of populations is restricted to near neighbors. The Mantel test computes a correlation between two *n* by *n* distance matrices. Lines of a pairwise genetic distance semimatrix ($F_{ST}/(1 - F_{ST})$) (Rousset, 1997) were permuted against a second distance semimatrix (geographical or maturity-zone distance based on historical origin) 1000 times to generate the distribution of the rank correlation coefficient and to test the null hypothesis of independence between the two distances. Data for 11 accessions, that is, 55 pairs of populations, were used in Mantel tests.

To examine distance (km) and maturity zone independently, partial matrix correlation versions of the Mantel test were performed using Phylogeographer 1.0 (Buckler, 1999) for genetic distance vs. kilometers while maturity zone was held constant, and genetic distance vs. maturity zone while kilometers was held constant.

RESULTS

Descriptive Statistics

The total number of alleles discovered for the 20 SSR loci was 130, with the number of alleles per locus ranging

from 2 to 13 (Table 2). Missing data amounted to 1.9% of the total 9220 data points (461 plants by 20 markers), including true null alleles and failed PCR amplifications. The mean number of alleles per locus was 3.17 within accessions 1 to 47 (Table 3), and 3.20 for the pool of 12 inbreds. Only 14 of the 130 alleles were restricted to a single accession, three belonging to flint types (two in the Northern Flints and one in Falconer) and 11 belonging to Corn-Belt Dent types (nonSouthern Dent and noninbred). The number of alleles is dependent on sample size, which must be taken into account when making comparisons between accessions. The flint Longfellow ($n = 21.10$) had relatively few alleles [number of alleles (A) = 2.65], as did the Northern Flints ($n = 5.65$, $A = 2.60$). Longfellow, Falconer, and the Northern Flints (total $n = 33.55$) contained a total of 90 of 130 alleles, whereas the pool of 12 inbred lines contained 64 of 130 alleles.

Gene diversity, or expected heterozygosity under random mating, was lowest in Longfellow (gene diversity, D, = 0.33) and the other Northern Flints ($D = 0.42$), and highest in the pooled inbreds ($D = 0.61$), with a mean $D = 0.53$ for open-pollinated accessions (Table 1, Entries 1–47). For the majority of accessions, the observed heterozygosity was less than expected, indicating a tendency toward inbreeding within a population. The mean coefficient of inbreeding (f) was 0.10 (Table 3). The most extreme deviations from random mating were observed in Polar Dent ($f = 0.30$) and Falconer ($f = 0.25$) (Table 3).

Partitioning of Variation

For F-statistics analyses, statistically significant, small to moderate deviations from random mating of individuals within accessions were observed (Tables 4 and 5). Mean values of 0.08 and 0.09 for F_{IS} were consistent with the estimated mean coefficient of inbreeding of 0.10 (Table 3). Approximately 0.15 reduction in heterozygosity was found among accessions for Entries 1 to 47 ($0.14 < F_{ST} < 0.16$, $P < 0.05$); that is, on average, 85% of the total molecular genetic variation was shared among them as it would be if there were random mating among all 47 accessions (Table 4). When accessions with the same name were grouped, the six groups shared an average of 96% of their variation ($0.02 < F_{TOT} < 0.06$, $P < 0.05$) (Table 5).

Table 4. F statistics and 95% confidence intervals from 1000 bootstrap replicates for 47 corn accessions based on 20 SSR loci.

	F_{IS}†	F_{IT}‡	F_{ST}§
Mean¶	**0.09**	**0.23**	**0.15**
Lower bound	**0.04**	**0.17**	**0.14**
Upper bound	**0.16**	**0.29**	**0.16**

† The mean reduction in heterozygosity within individuals due to nonrandom mating within accessions.
‡ The mean reduction in heterozygosity within individuals due to nonrandom mating within and among accessions.
§ The mean reduction in heterozygosity within accessions due to nonrandom mating among accessions.
¶ Entries 1 through 47 in Table 1.

Table 5. F statistics and 95% confidence intervals from 1000 bootstrap replicates for 22 corn accessions placed into six groups based on 20 SSR loci.

	F_{IS}†	F_{IT}‡	F_{SUB}§	F_{TOT}¶
Mean#	**0.08**	**0.18**	**0.11**	**0.04**
Lower bound	**0.02**	**0.13**	**0.10**	**0.02**
Upper bound	**0.14**	**0.24**	**0.13**	**0.06**

† The mean reduction in heterozygosity within individuals due to nonrandom mating within accessions.
‡ The mean reduction in heterozygosity within individuals due to nonrandom mating within and among accessions.
§ The mean reduction in heterozygosity within accessions due to nonrandom mating within groups.
¶ The mean reduction in heterozygosity within groups due to nonrandom mating among groups.
# Groups were Clarage, Golden Glow, Krug, Lancaster Sure Crop, Midland, and Reid Yellow Dent. Subgroups were accessions of these.

Genetic Relationships

The Fitch-Margoliash tree (Fig. 1) partly confirmed predicted genetic structure among the accessions. The most strongly supported relationships were the distinct clustering of the Northern Flints, Longfellow (flint), and Falconer (flint) away from all other accessions, with Lancaster Sure Crop being their closest relative. The four Midland accessions clustered together, as did many of the Reid Yellow Dents and their derivatives (Funk's Yellow Dent, Iodent). The extremely low bootstrap values throughout the base of the tree indicated no evidence of higher-order groups within the nonflint germplasm.

GRIN Data

Pollen-shed data for 27 accessions (Table 3) were collected from one location in 1 yr (Ames, IA, 1987) so different accessions can be directly compared. The earliest flowering cultivars were Minnesota 13 (56 d) and Longfellow (61 d), both strains adapted to Ontario, Canada. The latest flowering cultivars were Bowman's Cole Creek (80 d) and Midland (81 d), both adapted to eastern Kansas. Days to pollen shed were compared with maturity zone of strain origin for the 27 accessions and a north/south cline was evident (Table 3). A significant correlation was found between days to pollen shed and maturity zone of the strain's origin ($r = 0.76$, df = 25, $P < 0.01$).

Isolation-by-distance

Statistically significant correlations were found between genetic distance and geographical distance ($r = 0.54$, $P = 0.04$), and between genetic distance and maturity zone distance ($r = 0.33$, $P = 0.03$). Partial matrix correlations between genetic distance and kilometers, holding maturity zone constant ($r_{12.3} = 0.51$, $P = 0.02$); and genetic distance and maturity zone, holding kilometers constant ($r_{12.3} = 0.27$, $P = 0.06$), suggested that distance in kilometers explained most of the observed isolation-by-distance. When Longfellow was removed from the analysis, the significant correlation between genetic and geographical distance disappeared ($r = 0.17$, $P = 0.27$), as did the significant correlation between genetic distance and maturity zone distance ($r = 0.20$,

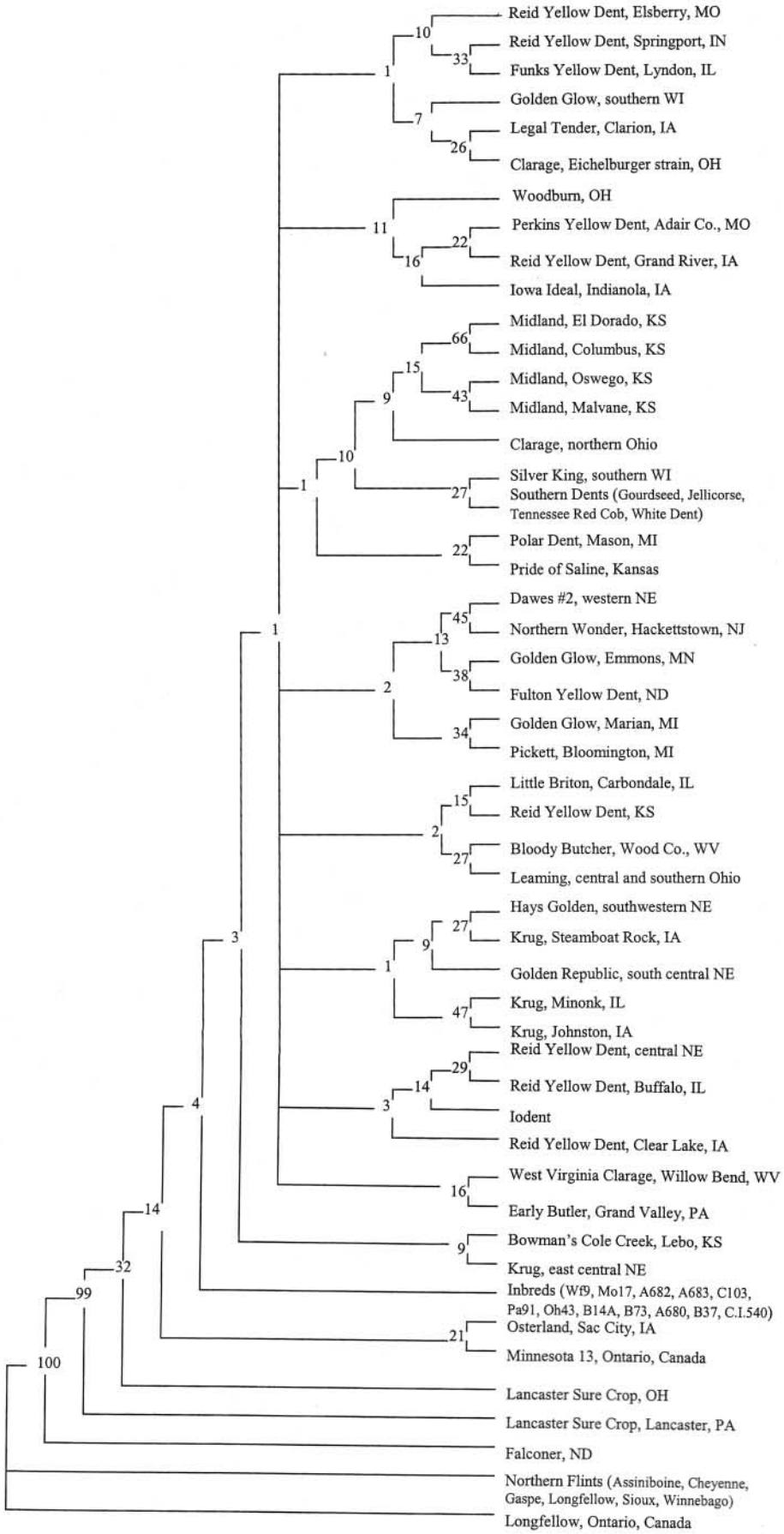

Fig. 1. Fitch-Margoliash tree of Corn-Belt Dent accessions based on 20 SSR loci. Values indicate the number of times a particular node was supported in 100 bootstrap replicates.

$P = 0.17$). Longfellow originated in the extreme eastern USA (Massachusetts; Table 1).

Relative distances among Longfellow and the other cultivars were complexly related. For example, Longfellow and Minnesota 13 displayed relatively large genetic and geographical distances, whereas Longfellow and Falconer were genetically closely related but quite distant geographically. Falconer originated in North Dakota but specifically lists Indian flint in its background (Table 1). Longfellow and Lancaster Sure Crop were close geographically but not closely related genetically.

DISCUSSION

Genetic Variation

Optimal sample size for a study such as this is an important consideration. With finite financial resources, the number of data points collected must be allocated across populations, individuals, and loci. Two sources of error, experimental sampling and genetic drift, contribute to variation in allele frequency estimates among closely related accessions. We assume that genetic drift has led to divergence among closely related accessions, strains, and cultivars. We have no a priori evidence that any of the SSR loci that we genotyped is correlated with any favorable phenotype. Therefore, changes in allele frequencies at these particular markers because of natural or human selection may be rare.

Fewer individuals per population and more genetic markers are a generally preferred experimental design for many population genetics studies (Baverstock and Moritz, 1996). Other corn population genetic studies have sampled ≈5 to 30 plants (e.g., Doebley et al., 1985, 23 isozyme markers; Smith, 1986, 21 isozyme markers; Rebourg et al., 1999, 23 restriction fragment length polymorphism (RFLP) markers, and sometimes ≈100 plants (Reedy et al., 1995, 28 isozyme markers; Labate et al., 1997, 82 RFLP markers). For Northern Flint and Southern Dent germplasm, we sampled single plants from several accessions and pooled the genotypic data. These data were used as outgroups rather than to describe Northern Flint or Southern Dent germplasm in detail. Single plants sampled from inbreds were assumed to be valid representatives of each inbred. Inbreds are occasionally heterozygous at SSR loci, but the heterozygosity of an inbred line within a given source has been found to be <5%, and between sources <8% (Gethi et al., 2002).

Molecular markers must be highly polymorphic for population genetics studies. Simple sequence repeat markers have been found to be more polymorphic than RFLPs and isozymes in corn (Smith et al., 1997; Pejic et al., 1998; Senior et al., 1998). Thirty-three corn inbreds reliably clustered into known pedigrees on the basis of 27 SSR loci (Pejic et al., 1998). As few as five SSR loci were sufficient to provide unique fingerprints for 94 elite inbreds (Senior et al., 1998). Matsuoka et al. (2002) found SSR loci to be reliable for measuring intraspecific variation in 14 landraces (including four subspecies) and 101 inbreds of *Zea mays*.

The purpose of our study was to obtain a broad view of Corn-Belt Dent germplasm, focusing on genealogical, ecological, and human influences rather than exploring particular accessions or loci in detail. Amount and distribution of diversity were of primary interest, and during the course of data collection, we concluded that our sample sizes of 5 to 23 plants per accession and 20 SSR loci were quite adequate. F_{ST} estimates of the 47 accessions for a random initial subset of the data consisting of 249 individuals and nine SSR loci (mean F_{ST} = 0.14, 95% CIs = 0.11, 0.16) were similar to final estimates for 20 loci and 439 individuals (mean F_{ST} = 0.15, 95% CIs = 0.14, 0.16). In comparing accessions with different sample sizes, we did not gain much additional information by sampling more individuals per accession. The mean number of alleles per locus across all 47 accessions was 6.5 and that within accessions was 3.2. Increasing mean sample size from 7 to 21 did not greatly change mean A or mean D. For 40 accessions with $n \leq 8$ (mean $n = 7.18$), mean $A = 3.14$ and mean $D = 0.54$; whereas for seven accessions with $n > 18$ (mean $n = 20.55$), mean $A = 3.36$ and mean $D = 0.49$.

A small percentage (11%) of alleles was restricted to one accession. These were spread across 14 different accessions and 11 different loci, indicating that unique alleles were not generally responsible for differentiating accessions. Flint germplasm was distinct from nonflint germplasm, not because it contained different alleles but because it lacked a large portion of alleles that were frequently shared among the other accessions. Flint germplasm, although it has a narrower genetic base, exhibited more variation among accessions compared to dent; mean $F_{ST} = 0.35$ for 20 accessions of Northern Flints and Flours genotyped at 14 SSR loci (González Ugalde, 1997).

The set of 12 inbred lines was relatively diverse ($D = 0.61$) compared with open-pollinated populations ($D = 0.53$) because allele frequencies had become more evenly distributed. The sample of 12 inbreds contained 64 of 130 or 49% of the total number of alleles found in the other 449 sampled individuals. This suggests that a large fraction of the molecular genetic variation from open-pollinated populations was captured in newly developed inbred lines in the 1920s to 1940s. It is stressed, however, that the open-pollinated populations in our study were not a random sample of Corn-Belt Dent germplasm but are known to have been important sources of many modern inbreds.

Mean coefficients of inbreeding and F_{IS} estimates suggest that the accessions were slightly more homozygous than if they were random-mating. This was statistically significant in bootstrap tests of F_{IS} (Tables 4, 5), significance being that 95% CIs did not overlap with zero. Inbreeding has been observed in other corn populations (Kahler et al., 1986; Dubreuil and Charcosset, 1998; Labate et al., 2000) and may reflect a tendency toward positive assortative mating for flowering time. Intraaccession variation in maturity creates the risk of losing alleles, particularly if a season is unusually short.

Interpretation of Genetic Structure

Each accession that we genotyped was associated with one or two origins, one based on its pedigree (historical

origin) and the other based on where it was collected and presumably most recently adapted (strain origin). Within Corn-Belt Dent cultivars, different strains were selected by farmers for adaptation to local conditions. The USDA 1936 Yearbook of Agriculture stated, "Variety names of corn mean less than those of almost any other field crop" (Jenkins, 1936). This statement was based on empirical evidence that had been gathered by Agricultural Experiment Stations throughout the USA. For example, yield trials in Kansas from 1903 to 1909 compared seven cultivars of Kansas-produced seed to the same cultivars from seven other states. In 39 of 40 pairwise comparisons, the Kansas-grown seed outyielded the other, and similar results were found for within-state variation between localities (Cunningham and Wilson, 1921). Experiment stations in other states similarly reported that there was more variation in yielding ability within a cultivar than between cultivars, and that locally improved and adapted seed was highly preferred (Nebraska: Lyon, 1904; Connecticut: Jones et al., 1924; Minnesota: Dunham, 1928; Iowa: Hughes et al., 1929). In one experiment station bulletin, a "variety" was stated to be "a very complex thing... fairly uniform as regards only certain gross characters such as color" (Olson et al., 1927). It was recommended that imported seed corn be secured from an environment similar to that within which it would be grown (Cunningham and Wilson, 1921). For example, it was reported that, in Iowa, a satisfactory yield could be obtained from a sample of corn when grown either to the east or west of its point of origin (Hughes et al., 1929). Because of the importance of local adaptation, farmers were warned against frequently obtaining new seed from outside their immediate locality (Cunningham and Wilson, 1921). In the early twentieth century in Iowa, there were estimated to be hundreds of strains of Reid Yellow Dent and dozens of strains of Silver King (Hughes et al., 1929). It is within this context that our genetic evidence must be interpreted.

Because corn originated in the tropics, it has experienced selection pressure in North America for early maturity. Our observation that pollen-shed date, a quantitative measure of relative maturity, was significantly correlated to strain origin, suggests that genetic differences among the strains that reflect their original adaptation to local conditions still exist. This is in spite of the fact that the accessions have been stored in the same location (Ames, IA) for several decades and are possibly undergoing some natural selection and genetic drift during periodic regenerations. We postulated that there have been three major factors influencing the original genetic constitution of the accessions: (i) pedigree, (ii) migration and gene flow from east to west or south to north, or both, and (iii) selection for local adaptation. One of the strongest selective pressures was toward increasingly earlier maturity as the crop moved northward.

One drawback to pedigree information is that it is inclusive, assuming that historical accounts are accurate, but not exclusive. For example, many cultivars have purportedly been derived from Reid Yellow Dent, but it cannot be assumed that a particular cultivar was not derived from Reid Yellow Dent simply based on the lack of such a statement. Beyond narrative accounts, the consensus Fitch-Margoliash tree is one way to infer pedigrees. The tree displayed well-supported structure on a gross scale (flint vs. nonflint) but little fine-scale resolution. This lack of fine-scale resolution was supported by *F*-statistics; on average, most of the molecular genetic variation was shared among Entries 1 to 47.

When comparing all germplasm, many relationships that might have been expected were not visible. For example, Clarage and West Virginia Clarage accessions did not cluster; nor did Golden Glow accessions. Reid Yellow Dent and its known derivatives (Funk's Yellow Dent, Krug, Osterland, Pickett, and Iodent) did not form a single distinct cluster. Previously recognized major groups were supported when a subset of the data was analyzed. Lancaster, Reid Yellow Dent, and Midland accessions (13 in total) formed three distinct clusters even though they included differently adapted strains.

The trees cannot be interpreted too rigorously, because each tree is rarely a unique solution and only reflects one representation of the data. We conclude that pedigree has strongly influenced the genotypes of the accessions, but cultivars are so closely related that they become increasingly less distinct when more strains are included in the analysis, the exception to this being the divergence of the flints. These findings are consistent with what is known about the history of the germplasm. The flints were genetically isolated from the dents in the USA for >2500 yr, hence their extreme divergence. Regarding the apparent high levels of admixture among open-pollinated Corn-Belt Dent cultivars, there exist numerous descriptions of farmers trading and mixing seed, and observations that particular cultivars frequently became widely distributed, for example, after winning a World's Fair competition (Wallace and Brown, 1956).

Although the flint-dent dichotomy and pedigree of the cultivar were apparent in the data, there was no evidence for two broad groupings of Reid and Lancaster germplasm, nor of Wf9 and C103 within the Corn-Belt Dents. Principal components analysis of the variance-covariance matrix of arcsine-transformed allele frequencies distinguished the same two groups as the Fitch-Margoliash tree: Longfellow, the Northern Flints, and Falconer vs. all other accessions. The Southern Dents that we sampled, unlike the Northern Flints, were not distinct from the Corn-Belt Dents.

Intercultivar genetic differentiation based on locally adaptive requirements, although inferred from pollen-shed data and deduced to be present from historical accounts of yield tests, was not evident at the molecular genetic level. Mantel tests of isolation-by-distance were not significant when Longfellow was excluded from the analysis. Therefore, the data did not show evidence of migration or new genetic adaptations as the dent types gradually moved northward, but rather, crossing of Southern Dents to native Northern Flints to confer early maturity. Farmers moving from Illinois, Iowa, and Wisconsin were not successful in adapting Midwestern Dent

cultivars they brought with them to more northern climates until they crossed them to flints (Hays, 1904; Dunham, 1928).

It is not surprising that the 20 SSR loci that we genotyped did not reveal evidence of selection and adaptation in our study. Genetic differences among cultivars that confer adaptedness may constitute only a minor portion of total polymorphism, and may take great efforts to discover. Pairs of corn inbreds with small genetic differences from each other (≈12% genome-wide as estimated by molecular markers) were found to significantly differ in agronomic performance (Troyer and Rocheford, 2002). Data from quantitative trait loci (QTL) studies suggest that days to pollen shed is polygenically inherited (Kozumplik et al., 1996; Lin et al., 1995). Approximately 30 QTL for heat units to pollen-shed (qhupol) are compiled in the MaizeDB, and they are widely distributed across the corn genome. Study of finely spaced, highly polymorphic genetic markers around one or more of these QTL may reveal loci involved in local adaptation, because population history influences the entire genome in similar ways, but selection acts in specific ways on smaller genomic regions (Pritchard, 2001). This principle will provide a framework within which further sampling and study of the Corn-Belt Dent populations can be performed.

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